

# **LIPOSOMES FOR TREATMENT OF INFLAMMATORY DISEASES**

**Thesis**

**Varsha Komalla**

**2020**

Doctor of Philosophy

Graduate School of Health, Discipline of Pharmacy:

University of Technology Sydney

## **CERTIFICATE OF ORIGINAL AUTHORSHIP**

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This research is supported by the Australian Government Research Training Program.

Signature of Student:

Production Note:  
Signature removed prior to publication.

Date: 7 October 2020

## ACKNOWLEDGEMENTS

I would like to express my heartfelt thanks to my primary supervisor Dr. Mehra Haghi who has been supportive from the starting day of my Ph.D. candidature and provided me untold opportunities that helped me to become as a progressive and critical thinking student in research. I am also thankful to my co-supervisor Prof. Brian Oliver for his extended collaborative support and supervision.

I would like to thank A/Prof Hui Chen for her co-supervision and support in investigating the efficacy of liposomes in the *in vivo* models of inflammation. I would also like to thank Dr. Jeremy Chan, Gerard Li and Baoming Wang for their assistance during the mouse model of the high-fat diet study. I would like to thank Dr. Shafagh Waters for her expertise and collaborative work in performing experiments on primary epithelial cells. I would also like to thank Dr. Philip Kwok for his help in performing and analyzing the formulation data conducted at the University of Sydney. I would like to greatly acknowledge my friend and colleague, Raj Allam for his assistance in completing the *in vivo* model of respiratory inflammation and for his immense support and pieces of advice throughout my Ph.D.

I would like to especially thank Mercedes, Sarah, Razia Zakarya, Dia Xineki, Bishwajit who have helped me around in the lab on countless occasions. I would also like to acknowledge Fiona Ryan and Lalit Overlunde for their support in Ernst's facility during the mice studies.

It's my fortune to gratefully acknowledge the support of my friends Behjat, Senani, Pooja, and Dr. Kamal Dua for their support and generous care throughout the

research tenure. They were always beside me during the happy and hard moments to push me and motivate me. Big thanks to all my colleagues and friends particularly Newsha, Jack, Gabrielle for their co-operation and support and company on the weekends.

I would like to express my gratitude to the University of Technology Sydney (UTS) and Graduate School of Health (GSH) for supporting me throughout my studies with the UTS International Research Scholarship and UTS President's scholarship.

Lastly, this journey wouldn't have been complete without my parents and brother who mean a lot to me for showing selfless love and supporting me in every situation. I would like to thank my loving husband, Vinay from the bottom of my heart for his endless support during the candidature.

# **PUBLICATIONS AND PRESENTATIONS DURING CANDIDATURE**

**Book Chapter:** Liposomes in treatment of chronic respiratory conditions

Varsha Komalla, Mehra Haghi

The book entitled “Targeting chronic inflammatory lung diseases using advanced drug delivery systems.” commissioned by Elsevier

**Manuscript under submission:** The potential for phospholipids in treatment of airway inflammation: an unexplored solution

Varsha Komalla, Mehra Haghi, Meenu Mehta, Fatima Achi & Kamal Dua

**Journal:** Current molecular pharmacology

**Manuscript accepted:** A Phospholipid-Based Formulation for the Treatment of Airway Inflammation in Chronic Respiratory Diseases

Varsha Komalla; Venkata Sita Rama Raju Allam; Philip Chi Lip Kwok; Behjat Sheikholeslami; Louisa Owen; Adam Jaffe; Shafagh A Waters; Brian Gregory Oliver; Hui Chen; Mehra Haghi

**Journal:** European journal of pharmaceuticals and biopharmaceutics

**Manuscript under revision:** Impact of a Cargo-less Liposomal Formulation on Dietary Obesity-Related Metabolic Disorders in Mice

Varsha Komalla, Behjat Sheikholeslami, Gerard Li, Bishwajit Bokshi, Yik Lung Chan, Alison Ung, Brian Gregory Oliver, Hui Chen, Mehra Haghi

**Journal:** International Journal of Molecular Sciences

### **Oral Presentations**

Quercetin and resolvin D1 liposomes as a novel therapy for steroid-resistant airway diseases, Drug Delivery Australia, Wollongong, October 2017.

Liposomal formulation of quercetin and resolvinD1 for treatment of steroid-resistant airway inflammation, TSANZ, Adelaide, March 2018.

Novel phospholipid based formulation to reduce airway inflammation, NAME, Sydney, November 2019.

### **Poster Discussion**

The Effect of Novel DOPC Liposome on Preventing High-Fat Diet-Induced Metabolic Disorders in Mice

Varsha Komalla, Yik Lung Chan, Gerard Li, Baoming Wang, Brian G Oliver, Hui Chen, Mehra Haghi

University of Technology Sydney 3 Minute Thesis (3MT) 2017

University of Technology Sydney 3 Minute Thesis (3MT) 2018

# TABLE OF CONTENTS

Liposomes For Treatment Of Inflammatory Diseases.....	i
Certificate of original authorship.....	iii
AcknowledgEments .....	iv
Publications and Presentations During Candidature.....	vi
Table of Contents.....	viii
List of Figures .....	xiv
List of tables.....	17
List of Abbreviations.....	19
Abstract.....	28
Chapter 1 .....	31
General Introduction .....	31
Chapter 1a .....	32
The Potential for Phospholipids in the Treatment of Airway Inflammation: an Unexplored Solution.....	33
1. Inflammation in airway diseases.....	35
1.1 Mechanisms of defense in the lungs .....	35
1.2 Chronic pulmonary inflammation .....	37
1.3 Approaches to investigate lung inflammation .....	41
2. Current therapies and areas of unmet need in the treatment of chronic respiratory diseases.....	42
2.1 Corticosteroids .....	42
2.2. Biologics .....	44



2.3. Other therapies.....	45
3. Phospholipids .....	46
3.1. Phospholipid chemistry .....	47
3.2. Roles of endogenous phospholipids.....	48
3.3. Phospholipids in drug delivery.....	51
3.4. Therapeutic application of phospholipids in the treatment of inflammatory diseases.....	53
4. Discussion .....	68
5. Conclusion.....	70
Chapter 1b .....	72
1. Phospholipid-based formulations .....	74
1.1 Ethosomes .....	74
1.2 Phytosomes .....	74
1.3 Phospholipid based micelles .....	75
1.4 Intravenous Lipid Emulsions .....	76
1.5 Liposomes .....	76
2. Preparation of liposomal formulations .....	77
3. Barriers and challenges to pulmonary drug delivery.....	78
3.1 Fundamentals of Particle Deposition in the Lung .....	79
4. Physicochemical characterization of inhalable liposomes .....	81
4.1. Evaluation of aerodynamic properties .....	82
5. Devices to deliver liposomal formulations directly to the airways .....	82
5.1. Nebulizers .....	82

5.2. Pressurized metered-dose inhalers .....	85
5.3. Dry powder inhalers .....	86
6. Liposomal active vs passive targeting .....	86
6.1. Passive targeting.....	87
6.2. Active targeting.....	89
7. Preclinical studies on liposomes for treating respiratory diseases.....	92
7.1. <i>In vitro</i> studies .....	92
7.2. <i>In vivo</i> studies.....	93
8. Clinical assessment of liposomal formulations in respiratory diseases .....	95
8.1. Arikace .....	95
8.2. Liposomal ciprofloxacin .....	98
9. Conclusions.....	100
Hypotheses and aims .....	102
Chapter 2 .....	104
A Phospholipid-Based Formulation for the Treatment of Airway Inflammation in Chronic Respiratory Diseases.....	104
1. Introduction.....	108
2. Materials and Methods .....	111
2.1 Materials .....	111
2.2 Preparation of UTS-001 liposomes .....	112
2.3 Characterisation of liposomes.....	112
2.4 <i>In vitro</i> studies.....	115
2.5 <i>In vivo</i> studies .....	118

2.6 Statistical analysis .....	119
3. Results .....	120
Physical characteristics of UTS-001 .....	120
UTS-001 non-toxic doses were determined <i>in vitro</i> and <i>in vivo</i> .....	121
UTS-001 enters airway epithelial cells mainly via caveolae-mediated endocytosis .....	121
UTS-001 treatment attenuates TNF- $\alpha$ -induced IL-6 cytokine levels in primary airway epithelial cells.....	122
UTS-001 attenuates airway eosinophilic inflammation and AHR in a murine model of allergic airways disease.....	122
4. Discussion .....	123
5. Conclusions.....	129
Conflicts of interest.....	129
Author contributions .....	129
Acknowledgments .....	131
Figures and figure legends.....	132
Chapter 3 .....	148
Pathophysiology of Obesity and the Potential Use of Phosphatidylcholine for Management of Obesity.....	148
Pathophysiology of Obesity and use of Phosphatidylcholine for the disease management.....	149
Abstract .....	149
1.1 Introduction.....	149
1.2 Current treatment for obesity.....	150
1.3 Role of adipose tissue in obesity .....	152

1.4 Role of macrophages in obesity .....	152
1.5 Role of essential lipids in the prevention of obesity .....	153
1.6 Conclusions .....	155
Chapter 4 .....	156
Impact of a Cargo-less Liposomal Formulation on Dietary Obesity-Related Metabolic Disorders in Mice .....	156
1. Introduction.....	158
2. Results .....	160
2.1. <i>In vivo</i> studies.....	160
2.2. Effect of UTS-001 on uptake of 2-(N-(7-nitrobenz-2-oxa-1, 3-diazol- 4-yl) amino)-2 deoxyglucose (2-NBDG), number and diameter of adipocytes <i>in vitro</i> .....	174
3. Discussion .....	176
4. Materials and Methods .....	183
4.1 Animal experiments.....	183
4.2 Cell culture studies and Cellular 2-NBDG uptake.....	185
4.3 Statistical analysis .....	186
Abbreviations.....	187
Supplementary Methods.....	189
Appendix A.....	190
Chapter 5 .....	193
General Discussion .....	193
General Discussion .....	194
Strengths of the current study .....	196

Limitations of the thesis .....	197
References.....	199

# LIST OF FIGURES

## Chapter 1a

Figure 1: General structures of glycerophospholipids; $R^1$ , $R^2$ indicate variable acyl groups with fatty acid hydrophobic chains.....	48
---	----

## Chapter 1b

Figure 1. Application of phospholipids in drug delivery.....	76
Figure 2. Illustration of the jet nebulizer.....	82
Figure 3. (a) A concept of vibrating mesh nebulizer technology, (b) Mesh assembly and (c) Commercially available vibrating mesh nebulizer from Aerogen Ltd.....	83
Figure 4. Illustration of pressurized metered-dose inhaler.....	84

## Chapter 2

Figure 1. Physical characteristics of UTS-001.....	132
Figure 2. Evaluation of <i>in vitro</i> and <i>in vivo</i> biocompatibility of UTS-001.....	136
Figure 3. <i>In vitro</i> uptake of UTS-001 by BEAS-2B cells.....	138
Figure 4. Anti-inflammatory properties of UTS-001 in the culture of primary nasal epithelial cells.....	140
Figure 5. Efficacy of UTS-001 in an <i>in vivo</i> model of airway inflammation....	141

Figure 6. Lung function and airway hyperresponsiveness of lungs in response to increasing doses (0-50 mg/mL) of methacholine using Flexivent.....	142
Figure 7A. The pictures (magnification, 40X) represent the DifQuik stained cytopins prepared from the bronchoalveolar lavage fluid of PBS, OVA and OVA + UTS-001 treated mice respectively. ....	143
Figure 7B. Inflammatory responses in bronchoalveolar lavage of mice showing total cell numbers and differential counts.....	145
Figure S1. DSC thermograms of the freeze-dried UTS-001 with trehalose ....	146

## Chapter 4

Figure 1. a) An intraperitoneal glucose tolerance test (IPGTT, glucose 2g/kg) after 4 weeks of treatments. b) Area under the curve (AUC) of the IPGTT in (a) ...	164
Figure 2. Liver mRNA expression of inflammatory markers a) TNF- $\alpha$ , b) IL-6, c) MCP-1, d) TLR-4. e) IL-1 $\beta$ . Percentage macrophage number as identified by f) F4/80 and g) CD68 and representative images for stains (h & i) .....	167
Figure 3. Fat mRNA expression of inflammatory markers a) TNF- $\alpha$ , b) IL-6, c) MCP-1, d) IL-1 $\beta$ . e) Percentage macrophage number as identified by F4/80 and f) Representative images for stain .....	169
Figure 4. mRNA expression of liver glucose and lipid markers a) FOXO-1, b) GLUT-2, c) PPAR $\gamma$ , d) PGC-1 $\alpha$ . e) SREBP-1c, f) FASN, g) ATGL, h) CPT-1a.....	172
Figure 5. mRNA expression of a) CPT-1a, b) ATGL and c) PPAR $\gamma$ in fat.....	173

Figure 6. mRNA expression of a) UCP-1 and b) UCP-3 in brown fat.....	174
Figure 7. a) Insulin-mediated glucose uptake in mature adipocytes pretreated with PBS, Metformin and UTS-001. b) Number c) Diameter of mature adipocytes treated with PBS and UTS-001 .....	175
Figure A1. a) An intraperitoneal glucose tolerance test (IPGTT, glucose 2g/kg) after 4 weeks of treatments. b) Area under the curve (AUC) of the IPGTT in (a) .....	191



# LIST OF TABLES

## Chapter 1a

Table 1. Examples of common approaches and techniques to assess lung inflammation.....	41
--	----

Table 2. <i>In vitro</i> studies showing the anti-inflammatory potential of phospholipids in various diseases.....	60-64
--	-------

Table 3. <i>In vivo</i> studies showing the anti-inflammatory potential of phospholipids in various diseases.....	64-67
---	-------

## Chapter 2

Table 1. Stability of UTS-001 in terms of size, polydispersity index and zeta potential at day 0, 7, 14 and 30.....	<b>Error! Bookmark not defined.</b>
---	-------------------------------------

Table 2. Size, polydispersity index and zeta potential measurements of freeze-dried UTS-001.....	<b>Error! Bookmark not defined.</b>
--	-------------------------------------

Table 3. Summary of patient demographics.....	<b>Error! Bookmark not defined.</b>
---	-------------------------------------

## Chapter 3

Table 1. Classification of individuals based on BMI and probable risk of co-morbidities.....	149
--	-----

Table 2. Available drugs on the market for the treatment of obesity.....	151
--	-----

## Chapter 4

Table 1. Effects on anthropometric parameters of mice with HFD and UTS-001 treatment. ....	162
--	-----

Table 2. Lipid and glucose profiles in mice with HFD and UTS-001 treatment. ....	163
--	-----

Table A1. Anthropometric parameters of the mice treated with oil, DOPC liposomes, and DOPC+Cholesterol mixture.....	190
---	-----

Table A2. TaqMan® probe information (Life Technologies, CA, USA).....	192
---	-----

Table A3. SYBR® primer information (Sigma-Aldrich, MO, USA).....	192
--	-----

## **LIST OF ABBREVIATIONS**

PL: Phospholipids

COPD: Chronic obstructive pulmonary syndrome

CF: Cystic fibrosis

Th2: T Helper Cell Type 2

Th17: T Helper Cell Type 17

IL-4: Interleukin-4

IL-13: Interleukin-13

IL-5: Interleukin-5

IL-9: Interleukin-9

IL-17A: Interleukin-17A

IL-17E: Interleukin-17 E

IL-17F: Interleukin-17 F

IL-22: Interleukin-22

TNF- $\alpha$ : Tumor necrosis factor-alpha

IL-1 $\beta$ : Interleukin-1-beta

G-CSF: Granulocyte-colony stimulating factor

IL-6: Interleukin-6

CXCL1: C-X-C Motif Chemokine Ligand 1

Gro- $\alpha$ : growth-regulated oncogene-alpha

CXCL2: C-X-C Motif Chemokine Ligand 2

CXCL8: C-X-C Motif Chemokine Ligand 8

IL-8: Interleukin-8

(TGF- $\beta$ ): Transforming growth factor-beta

LTB4: Leukotriene B4

IP-10: Interferon-gamma inducible protein

I-TAC: Interferon-inducible T-cell chemoattractant

Mig: monokine-induced by interferon-c

CFTR: CF transmembrane conductance regulator

FEV1: forced expiratory volume

IgE: Immunoglobulin E

CXCR2: CXC chemokine receptor 2

p38 MAPK: p38 mitogen-activated protein kinase

GPLs: glycerophospholipids

PE: phosphatidylethanolamine

PI: phosphatidylinositol

PG: phosphatidylglycerol

PS: phosphatidylserine

PC: phosphatidylcholine

SM: Sphingomyelin

DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine

US-FDA: United States Food and Drug Administration

RDS: Respiratory distress syndrome

LPC: lysophosphatidylcholine

IP3: inositol triphosphate

DAG: diacylglycerol

AA: arachidonic acid

HETEs: hydroxy-eicosatetraenoic acids

EETs: epoxyeicosatrienoic acids

LCAT: lecithin-cholesterol acyltransferase

MCP-1: macrophage chemoattractant protein 1

PAF: Platelet-activating factor, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine)

OxPL: oxidized phospholipids

Nrf2: nuclear factor erythroid 2-related factor 2

HO-1: heme oxygenase-1

PPAR $\gamma$ : peroxisome proliferator-activated receptor gamma

NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells

TLR: Toll-like receptors

PAMPs: pathogen-associated molecular patterns

PRRs: pattern recognition receptors

PGE<sub>2</sub>: Prostaglandin E<sub>2</sub>

ICAM-1: Intercellular Adhesion Molecule 1

MPO: Myeloperoxidase

NAG: N-acetyl-glucosaminidase

SAPS: 1-stearoyl-2-arachidonoyl-sn-glycero-3-phospho-L-serine

HRV: Human rhinoviruses

CCL5: C-C Motif Chemokine Ligand 5

CXCL8: C-X-C Motif Chemokine Ligand 8

IFN-β: Interferon-beta

DOPG: Dioleoylphosphatidylglycerol

TPA: 12-O-tetradecanoylphorbol 13-acetate

POPG: Palmitoyl-oleoyl phosphatidylglycerol

RSV: Respiratory syncytial virus

CD14: Cluster of differentiation 14

COX-2: Cyclooxygenase-2

LPS: Lipopolysaccharide

MMP-1: Matrix metalloproteinases

PPC: Polyene Phosphatidylcholine

NAPE: N-acylphosphatidylethanolamine

HUVECs: human umbilical vein endothelial cells

VSMCs: rat vascular smooth muscle cells

DLPC: Dilinoleoylphosphatidylcholine

iNOS: inducible nitric oxide synthase

DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine

ERK: Extracellular signal-regulated kinase

*Actinobacillus pleuropneumoniae* (App)

MPS: mononuclear phagocyte system

SSMM: Sterically stabilized mixed micelles

MM: Mixed micelles

PEG: Polyethylene Glycol

LUVs: Large unilamellar vesicles

SUVs: Small unilamellar vesicles

MLVs: Multi-lamellar vesicles

pMDIs: pressurized metered dose inhalers

CFCs: Chlorofluorocarbons

IPF: Idiopathic pulmonary fibrosis

DPI: dry powder inhalation

ACI: Anderson cascade impactor

FPF: Fine particle fraction

EPR: Enhanced Penetration and retention effect

RES: Reticuloendothelial system

CAR: coxsackievirus–adenovirus receptor

RGD: arginine-glycine-aspartic acid

CTD-ILDs: Collagen Tissue Disease–associated Interstitial Lung Fibrosis

BOS: Bronchiolitis Obliterans Syndrome

MBSA: maleylated bovine serum albumin

O-SAP: O-steroyl amylopectin

PA: phosphatidic acid

PI3P: phosphatidylinositol 3-phosphate

APL: Apoptotic body like liposomes

LHLN: 6-lauroxyhexyl lysinate

NTM: nontuberculous mycobacterial infection

DPPC: dipalmitoylphosphatidylcholine



ALIS: Amikacin liposome inhalation suspension

MAC: Mycobacterium avium complex

ATS/IDSA: American Thoracic Society / Infectious Diseases Society of America

GBT: guidelines-based therapy

CFU: colony formation units

NCFB: non-CF Bronchiectasis

ORBIT: Once-daily Respiratory Bronchiectasis Inhalation Treatment

CRD: Chronic respiratory disease

18:1 liss-rhod-PE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N- (lissamine rhodamine B sulfonyl) (ammonium salt)

HBSS: Hank's balanced salt solution

ELISA: Enzyme linked immuno sorbent assay

TGA: Thermogravimetric analysis

DSC: Differential scanning calorimetry

MTT: (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)

DAPI: 4',6-diamidino-2-phenylindole

Flu-WGA: Fluorescent wheat germ agglutinin

PEEP: positive end-expiratory pressure

Rrs: Total Resistance

Ers: Total elastance

Crs: Compliance

Rn: Newtonian resistance

H: Tissue elastance

G: Tissue damping

AHR: Airway hyperresponsiveness

PC-PLC: Phosphatidylcholine-specific phospholipase C

P-chol: Phosphocholine

WAT: white adipose tissue

NEFAs: non-esterified fatty acids

SAA3: Serum amyloid A3

CDP-choline: cytidine 5'-diphosphocholine

HFD: High fat diet

CPT-1a: Carnitine palmitoyltransferase I

FASN: Fattyacid synthase

SREBP-1c: Sterol regulatory element-binding transcription factor 1

SLC2a2: Solute Carrier Family 2 Member 2

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

RT-PCR: Reverse transcription polymerase chain reaction

PBS: Phosphate buffer saline

TG: Triglycerides

FFA: Free fatty acids

PGC-1 $\alpha$ : Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

FOXO-1: Forkhead Box O1

DC: Deoxycholate

## Abstract

Inflammation is the body's defense mechanism to harmful stimuli such as microbial infection, allergy, stress or injury. Local or systemic inflammation is a major hallmark of several chronic diseases including asthma, obesity, diabetes, cystic fibrosis, cancer, irritable bowel syndrome and psoriasis among many others. Current treatments for the majority of these diseases do not address the underlying inflammation and in those conditions where inflammation is currently treated, there are several areas of unmet need where inflammation is not resolved with current medications or several side effects occur as a result of the treatment.

Phospholipids (PLs) are amphiphilic molecules capable of forming self-assembling structures in water, making them suitable excipients in drug delivery systems such as liposomes, niosomes, solid lipid nanoparticles and phytosomes. Phosphatidylcholine (PC) is the most abundant PL and is a major component of biological membranes.

The biological activity of some natural PCs and their mixtures have been demonstrated in the literature and some PCs have shown to have immunomodulatory properties in inflammatory conditions such as ulcerative colitis, arthritis and multiple organ injury. In gastrointestinal inflammation, PCs administered orally have shown local therapeutic effects; however, reduction of systemic inflammation following the ingestion of PLs has not been demonstrated. In systemic inflammation, systemic administration of naturally occurring PCs has shown anti-inflammatory properties. Natural PCs are a heterogeneous mixture of components, therefore the immunomodulatory efficacy observed in the studies cannot be attributed to one single component of the mixture.

Hydrogenated natural PLs and synthetic PLs are identified in the “Inactive Ingredient Guide” by the food and drug administration (FDA). However, no study to date has investigated the therapeutic efficacy of pure synthetic PLs in form of a solution or a liposomal formulation for the treatment of inflammatory conditions. We report the first application of empty liposomes made up of synthetic PC as an anti-inflammatory treatment, in a murine model of airway inflammation and a murine model of systemic metabolic inflammation.

The 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cholesterol containing liposomes (referred to as UTS-001) were prepared using the thin-film hydration method and characterized in terms of size, charge and polydispersity index. Stability studies were performed for over two months. Furthermore, UTS-001 was successfully freeze-dried using trehalose and characterized as per above for long-term storage.

Asthma is a complex, chronic inflammatory respiratory disease characterized by shedding of the epithelium, airway smooth muscle hypertrophy and hyperplasia, mucus overproduction, and airway inflammation. Airway inflammation in response to environmental pollutants causes activation of epithelial cells. The release of inflammatory mediators and the influx of inflammatory cells into the airways have been attributed to the pathophysiology of asthma.

In this thesis, we have demonstrated that UTS-001 was taken up by airway epithelial cells in a time-dependent manner via caveolae-mediated pathway. Furthermore, *in vitro*, in the culture of primary epithelial cells isolated from cystic fibrosis and asthmatic patients, UTS-001 treatment caused a significant reduction in interleukin-6 (IL-6) levels following stimulation with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). The immunomodulatory properties of UTS-001 were further investigated in an ovalbumin model of allergic

airway inflammation. Treatment with UTS-001 resulted in reduced airway hyperresponsiveness demonstrated by improved lung function measurement and decreased eosinophil number in bronchoalveolar lavage.

Obesity is commonly referred to as a systemic low-grade inflammation, exemplified by increased inflammatory macrophage number in metabolic tissues (e.g. liver and fat), as well as increased circulating inflammatory mediators. Inflammation is a well-accepted mechanism underlying weight gain, insulin resistance, hyperglycemia, and dyslipidemia in obesity. Efficacy of UTS-001 in reducing weight and systemic inflammation was investigated in a murine model of a high-fat diet. Administration of UTS-001 (along with consumption of the high-fat diet) resulted in a reduction of high-fat diet-induced inflammatory mediators such as TNF- $\alpha$  and peroxisome proliferator-activated receptor-gamma (PPAR- $\gamma$ ) in the liver. Retroperitoneal fat, liver weight and body weight were reduced significantly in obese mice following treatment with UTS-001. Macrophage numbers were reduced in fat and liver and intraperitoneal glucose tolerance test demonstrated an improved glucose tolerance following administration of UTS-001 in the high-fat diet-fed mice. Furthermore, UTS-001 caused an increase in thermogenic marker, uncoupling protein-1 (UCP-1) in the brown fat which increases non-shivering thermogenesis that enhances fat burning. Our studies demonstrated that UTS-001 has the potential to be used in the treatment of obesity and diabetes.

In summary, the studies presented in this thesis demonstrate a suitable pharmaceutical formulation of UTS-001 with anti-inflammatory properties with the potential to be used as a treatment in addressing local or systemic inflammatory conditions.